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This study surveys attitudes and policies of representative organizations concerning the issue of alternatives to animal testing. Positions of organizations in the scientific community, the regulatory community, industry, and the animal welfare community are examined within the context of growing pressures to adopt new toxicity testing methodologies. The resulting issues which effect regulatory development are identified. The report has also been prepared as an information resource and guide to the relevant technical literature. Regulatory policy issues concerning the use of alternative toxicity tests are identified and discussed in the report.

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ALTERNATIVE METHODS FOR TOXICITY TESTING:

REGULATORY POLICY ISSUES

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Notice: The views and opinions of the authors expressed herein do not necessarily state or reflect those of the United States Government, the Environmental Protection Agency, or the National Institutes of Health.

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Summary

Alternative Methods for Toxicity Testing:
Regulatory Policy Issues

Recent progress in the science of toxicology, the high costs of traditional animal tests, the time requirements for the completion of tests, and socio-ethical concerns surrounding whole-animal testing have resulted in pressures to decrease dependence on animal testing in the screening and ranking of toxic chemicals, and to substitute alternative testing methods.

This study surveys attitudes and policies of representative organizations concerning the issue of alternatives to animal testing. An alternative test is defined as any procedure that (i) replaces currently used animal tests with non-animal tests, (ii) reduces the numbers of animals in presently used tests, and/or (iii) that refines tests to reduce the pain and suffering of the animals used. Positions of organizations in the scientific community, the regulatory community, industry, and the animal welfare community are examined within the context of growing pressures to adopt new toxicity testing methodologies. The resulting issues which effect regulatory development are identified. The report has also been prepared as an information resource and guide to the relevant technical literature.

While there is a diversity of opinion in each of the surveyed communities, some generalizations can be made. Among scientists there is a consensus that animal testing can provide needed information which is not provided by non-animal methods, but that, nevertheless, some reductions and refinements in animal testing can be accomplished. The animal welfare

community generally holds that not enough effort has been expended in searching for alternatives, and is much more optimistic about the prospects for replacing animal testing than is the scientific community. The regulatory agencies have begun to respond to concerns about animal tests by implementing policies to reduce requirements for some types of animal toxicity testing and by increasing the flexibility of their guidelines. Many chemical, pharmaceutical, and cosmetic manufacturers would like to see even more flexibility in the regulations, and official acceptance of less expensive, short-term testing methodologies. Others in industry maintain that traditional animal tests are reliable and provide a great deal of information about toxicity that would be difficult to obtain in other ways.

Regulatory policy issues concerning the use of alternative toxicity tests are identified and discussed in the report. These are:

1. Criteria for the evaluation and adoption of alternative toxicity testing methods.
2. Periodic review of toxicity testing methods.
3. Consistency of policy among federal agencies.
4. The relationship of federal regulations to international guidelines on the performance of toxicity testing.
5. Access to data relevant to alternative test development and dissemination of information.
6. Identification of potential alternative testing methods from studies of environmental effects of toxic chemicals.
7. The development of incentives for the transfer of technology from the laboratory to practical application.
8. Public dialogue about new toxicity testing schemes.
9. The possible passage of legislation that would require changes in toxicity testing procedures.

Timely and thoughtful attention to these issues will enable appropriate policy development on alternative tests and ensure the protection of human and environmental health.

I. INTRODUCTION

As many as 65,000 to 100,000 chemicals are now in use in American industry, and approximately 1000 are added each year.[1] The U. S. Environmental Protection Agency (EPA) has the major regulatory responsibility for chemicals under the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Formal data requirements have been established for pesticidal products submitted for registration under FIFRA, and toxicity data may be requested for chemical products submitted for Premanufacture Notification (PMN) under TSCA. The test data are evaluated by EPA to determine the potential hazards of these products for human, animal, and environmental health.

Animal testing has been heavily relied upon for the assessment of chemical products. Toxicity testing may begin with acute tests in animals to establish the degree of toxicity and to identify the organs at greatest risk. These tests may then be followed by further animal testing for subchronic and chronic effects. However, recent progress in the science of toxicology, as well as high costs, excessive time requirements for completion of tests, and socioethical concerns surrounding whole-animal testing, have resulted in pressures to decrease dependence on animal testing. Consequently, there is a growing need to develop faster, cheaper, and more effective alternative methods of toxicity testing.

For the purposes of this study, alternative test methods can be defined according to the concept of the "3 Rs" -- replacement, reduction,

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[1] Tilson, H. A., and Mitchell, C. L. 1984. Neurobehavioral Techniques to Assess the Effects of Chemicals on the Nervous System. Ann. Rev. Pharmacol. Toxicol. 24: 425-450. Maugh, T. M. 1978. Chemicals: How many are there? Science 199: 162.

or refinement.[2] That is, alternative methods are any that (i) replace presently used animal tests with non-animal tests, (ii) that reduce the numbers of animals in presently used tests, or (iii) that refine tests to reduce the pain and suffering of the animals used.

The aims of this study are the following:

- To alert the Office of Policy, Planning, and Evaluation (OPPE) to changing perceptions in the scientific community of the acceptability of alternatives to animal testing, and of the political pressures to effect change.
- To survey the policies within EPA, other regulatory agencies, and representative groups in interested communities concerning alternative testing.
- To provide a resource for OPPE risk managers who wish to quickly familiarize themselves with the scientific issues underlying the alternative test methods debate, and to guide them to the relevant literature.
- To test the ease of direct access to the technical literature for the purpose of identifying new approaches to alternative tests in toxicology.

For the communities directly affected by the alternative testing issue, the concerns are not new. Regulators, legislators, scientists, manufacturers, and animal welfare advocates have been struggling for a number of years over the appropriate use of alternative test methods in toxicity testing. Judging by the number of recent news reports, the level of public interest may also be increasing. There can be no doubt that concern about animal testing has emerged as an important issue of popular debate.

Among the pressures to decrease animal toxicity testing are scientific progress in toxicology that has led to the development of promising

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[2] Russell, W. M. S., and Burch, R. L. 1959. "The Principles of Humane Experimental Technique." London: Methuen.

new methodologies, the rising cost of animals, limited laboratory space, the need for speed in obtaining results about potentially toxic chemicals, legal requirements regarding the use and treatment of animals, and ethical considerations about the use of live animals. Opposing pressures include legal and regulatory requirements that specify particular animal tests for product safety testing, heightened consumer awareness of potential risks of exposure to toxic chemicals with resulting demands for more thorough product testing, and scientific uncertainty about the validity of alternative tests. It should be appreciated that all toxicity testing methods, including both animal and in vitro tests, share a common problem -- questions about the ability of the test results to be extrapolated to human health considerations.

Confusion in terminology has often hindered discussion of these issues. Many terms with ambiguous and overlapping definitions are commonly used. Table 1 defines some of the terms most commonly used in toxicity testing.

Alternatives to animal toxicity testing has become a timely issue. A number of recent legislative proposals have called for the regulation of animal use and experimentation (see Appendix B). If passed, these proposals would have an immediate impact on the use of animals in toxicity testing. Also, two major government studies on the issue of alternative methods to animal tests will soon be released. The Office of Technology Assessment (OTA) is presently conducting an assessment for Congress entitled "Alternatives to Animal Use in Testing and Experimentation." The National Academy of Sciences (NAS) is conducting an evaluation of the opportunities and limitations in the use of nonmammalian models in biomedical research for the National Institutes of Health (NIH). A

Table 1

Terms Employed to Describe Tests Used in Toxicology

Term	Definition
Test, Assay, Bioassay	Effectively synonymous terms which refer to any laboratory technique or method for measuring a toxicologic effect.
Whole-Animal Test, In Vivo Test	Tests performed in the intact, living animal.
In Vitro Test	A test performed in an environment outside of the living animal, e.g., tissue culture. In vitro tests may require living animals for their starting materials
Animal	This term has a clear taxonomic definition, but as used in toxicity testing, is often taken to mean only vertebrate animals. Invertebrates are certainly animals, but may be acceptable to animal welfare advocates as alternatives to "animal" tests.
Non-Mammalian	Of or referring to organisms that are not in the taxonomic class Mammalia.
Short-Term	This term has no precise definition, but in toxicology, usually means a period of days or weeks. Short-term tests include all acute toxicity tests, virtually all in vitro tests, and some whole-animal tests. Often used interchangeably, but is not synonymous, with alternative.
Long-Term	In toxicology, a period of months or longer.
Alternative Test	A toxicity test that meets the criteria of the 3 Rs (replacement, reduction, or refinement of whole-animal methods) as described in the text.
Acute Toxicity	Adverse effects occurring within a short time after a single administration (e.g., oral, dermal, inhalation), or multiple doses within 24 hours, of a toxic substance.
Subchronic Toxicity	Adverse effects occurring from continuous or repeated doses of a toxic substance over a period of approximately 90 days.
Chronic Toxicity	Adverse effects that occur after a long latency period or that are caused by prolonged and repeated exposure to a toxic substance. Chronic effects appear approximately 6 months or longer after first exposure.

study of short-term testing is also in preparation by the World Health Organization.

These studies are likely to receive serious congressional attention and to focus public debate. Pressure for regulatory reform could therefore develop rapidly. For example, a proposal to require a battery of short-term toxicity tests similar to the Minimum Premarket Dataset (MPD) test battery of the Organization for Economic and Cooperative Development (OECD) is under consideration as an amendment to the TSCA reauthorization bill.

An additional example of the type of legislative action which may be anticipated is provided by the NIH reauthorization bill, that was passed at the close of the 98th Congress, and then vetoed by the President. The bill directed NIH to establish a plan to investigate methods of research which (i) do not require the use of animals; (ii) reduce the number of animals used in research; or (iii) produce less pain and distress in such animals than methods currently in use. (This language is the same as the "3 Rs" definition of alternative methods cited earlier.) The bill also called for a plan to validate new methods that are developed, for the training of scientists in the use of validated methods, and for the establishment of an Interagency Coordinating Committee to assist the Director of NIH in the development of the plan.

The objective of all toxicity testing is to identify substances that are potentially hazardous to human, animal, and/or environmental health. Current testing protocols for new chemicals generally include tests for acute and chronic effects that often require the use of a large number of animals and that may take 1 to 2 years to complete. These are commonly referred to as "whole-animal" tests, and include both "short-term" and "long-term" tests.

A major research effort of modern toxicology is the development and evaluation of new alternative tests for assessing potentially hazardous chemicals. Particularly desirable are tests that can be completed quickly and cheaply. For example, many "in vitro" systems -- such as bacteria, tissue culture, and organ culture -- are being investigated as possible alternative tests. For some types of toxicity, in vitro tests have become standard components of testing regimes, in addition to or in place of whole-animal studies.[3]

Although many alternative methods consistent with the "3 Rs" definition are short-term and inexpensive, others are not. For example, epidemiological studies are strongly favored by the animal welfare community as alternatives to animal testing, but are clearly not short-term and are usually very expensive. It should also be clearly stated that, as reduction and refinement are defined above, an alternative test may employ whole animals. The two terms (alternative and short-term) are often inappropriately and interchangeably used. In the following survey, we have tried to use the same terms that the respective organizations have themselves used to describe their activities. (Refer to Table 1 for definitions of these terms).

A distinction is frequently made between "animal research" and "animal testing." As commonly used, the term "animal testing" refers to the use of animals to evaluate the toxicity of potentially hazardous substances and to establish dose levels for pharmaceuticals, while "animal research" is a broader term including the use of animals in a variety of

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[3] For example, a number of bacterial mutagenicity assays are now commonly used, such as the Ames/microsome mutagenicity test which measures the mutagenic effect of chemicals on the bacterium Salmonella. Another example is the sex-linked recessive lethal test in Drosophila (fruitfly), used in the National Toxicology Program's second tier screening protocol.

disciplines in basic biological and medical research -- for example, biochemistry, immunology, pharmacology, etc.

It is probably fair to say there is a consensus among toxicologists that while particular in vitro tests may become effective substitutes for whole-animal tests, in vitro testing can never completely replace whole-animal testing. This is because in vitro tests cannot hope to replicate the functional and structural complexity of the intact animal nor to preserve the diversity of mechanisms for toxicity and detoxification that exists in living organisms. At each succeeding level of biological organization new properties appear which are not evident or even present in less complex levels of organisms or systems. While less complex biological systems or organisms offer models of biological processes which can sometimes be used to establish priorities for further testing of chemicals, the pitfalls involved in extrapolating data from such tests are many. Pharmacokinetic factors which determine how much active chemical will reach the "receptors" for toxicity differ greatly as do the organisms' homeostatic, adaptive, and repair mechanisms which influence the expression of toxic effects. On the other hand, in vitro tests usually have more precisely defined toxic endpoints than whole-animal tests, and therefore are superior for the investigation of the basic cellular and molecular mechanisms of toxicity. As a tool for basic research in toxicology, in vitro systems have great value.

The problem with in vitro systems for toxicity testing for regulatory purposes is that each test can generally identify only a narrow range of toxic effects. It is therefore important to develop test schemata which build toward humans in both their biological complexity and toxicological characteristics. This has led to a preference for several tests employed

in combination as a "battery" of tests.

Although some in vitro tests have been developed that have high predictive value for particular kinds of toxic effects (e.g., mutagenesis), there is a risk perceived in some quarters that complete dependence upon the results of in vitro assays in toxicity testing would lead to an unacceptably high proportion of false negatives and/or false positives -- chemicals whose actual toxic potential is incorrectly identified. For the forseeable future, in vitro tests will perhaps be most effective in screening protocols rather than as the primary determinant of toxicity.

Unfortunately, there is no perfect alternative test, whether performed singly or in combination with other tests. This is, of course, also true for whole-animal tests. No toxicity testing protocol can be 100% effective. Extrapolating the information gained from the various types of toxicity tests to human and environmental health effects is the critical and most uncertain step in toxicity assessment.

Criteria for measuring the validity of alternative testing methods have been recently described.[4] An alternative test should be easy to standardize, so that data from different labs are consistent. It must be able to detect toxicity over a wide range of different chemical structures and target tissues. Further, the test should be able to provide information on the toxicity of complex mixtures, and to indicate whether recovery from toxic insult is possible. These criteria represent a set of standards that any biosassay, including animal tests and in vitro tests, ought to satisfy in order to produce useful information concerning the toxicity of a chemical. A valid test must allow extrapolation of data from the test to human and/or environmental health, and must be at

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[4] Dagani, R. 1983. Chemical and Engineering News, Oct. 31, p. 7-13.

least as reliable as existing whole-animal tests.

Changing perceptions surrounding the appropriate use of alternatives to animal toxicity testing, coupled with social and political factors, argue that regulatory practices may need reexamination with respect to the use of alternative tests. Consequently, a number of policy issues need to be addressed, including (i) the codification of a process for the critical appraisal of alternative tests, (ii) periodic review of toxicity testing protocols, (iii) the human and environmental health implications of changes in toxicity testing protocols, and (iv) the development of a decision framework for the best regulatory use of alternative toxicity tests.

II. PREVAILING ATTITUDES TOWARD ALTERNATIVE TOXICITY TEST METHODS

Numerous groups have interests that are affected by the development of alternative toxicity tests. Prominent participants in the debate are the animal welfare community — consisting of antivivisectionists, animal rights groups, and humane groups; industry -- consisting primarily of chemical, pharmaceutical, and cosmetics manufacturers and their trade associations; the not-for-profit scientific community -- composed of academic institutions, various professional associations, and government research institutions; and the legislative and regulatory community at the federal, state, and local levels. Condensed summaries of the opinions held by representative groups within these communities are given below. These summaries were developed following interviews and examination of publications and meeting transcripts (see Bibliography and Appendices). Except for specific quotations, the summaries represent the authors' interpretation of where different groups stand on the alternative testing issue.

Acute toxicity tests, especially the LD_{50} and the Draize eye irritancy tests,[5] have been the primary target of animal welfare organizations that hope to eliminate or reduce animal toxicity testing. Regulatory agencies and chemical manufacturers have therefore tended to emphasize their efforts to eliminate or reduce dependence on these tests. The following survey of attitudes reflects this emphasis on acute toxicity testing. However, the statements often contain language that can be interpreted as a general philosophy on alternative toxicity tests.

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[5] The LD_{50} (Lethal Dose-50%) is a test that measures the dose, in a single administration, at which 50% lethality is observed in a test group of animals. In the Draize test, test materials are placed directly into the eyes of test animals (rabbits) to measure eye irritancy.

A. The Regulatory Community

The regulatory community is responsible for protecting human, animal, and environmenal health from hazardous substances. Different federal regulatory agencies are responsible for regulating different kinds of products, and operate through the authority of a variety of statutes. The agencies have each promulgated a variety of test standards, guidelines, and rules that affect the conduct of toxicity tests. In addition, state and local governments may have testing requirements that differ from federal requirements in significant ways. Some states enforce stricter requirements than federal law requires. Because federal regulations have the largest impact on toxicity testing practices, the following discussion will focus on the activities of the federal government.

Environmental Protection Agency

The relevant offices within EPA, for the purposes of this analysis, are the Office of Pesticides and Toxic Substances (OPTS) and the Office of Research and Development (ORD). Within OPTS, the Office of Toxic Substances (OTS) is responsible for administering TSCA and the Office of Pesticides Programs (OPP) for administering FIFRA. Both offices have evolving positions on the use of alternative methods for toxicity testing. ORD has an extensive research program in toxicology including a major effort in alternative test methods development. The current positions on how alternative tests should be employed are summarized for each office.

(a) OTS. New guidelines for determining acute toxicity were published by OTS in October 1984, covering oral, dermal, and inhalation toxicity.[6] The new guidelines clarify important testing options for

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[6] Environmental Protection Agency. 1984. Acute exposure guidelines - notice of availability through NTIS. Fed. Regis. 49: 39911-39912.

companies introducing new chemicals and provide more latitude for using alternative methods. Novel approaches to the determination of acute toxicity are recommended to encourage a reduction in the use of animals.

The Agency guidelines state that to minimize animal testing, sufficient information about toxic effects may in some cases be obtained from previously determined toxicity test results of structurally related chemicals. When animal tests are requested, EPA identifies the "limit test" as a permissible substitute for the traditional LD_{50} test. In the "limit test" a single group of animals is given an appropriate dose of the test agent, and if no lethality is observed, then further testing is not pursued for acute toxicity using the LD_{50}. Sometimes a limit test can reduce tenfold the number of animals used.

To diminish the number of animals used, the Agency also recommends an estimated lethal dose. This can be calculated by extrapolation or interpolation of data from a small test group of experimental animals. However, it has been pointed out that substitution of the estimated lethal dose for the LD_{50} test may require larger safety factors to be applied to account for the greater level of uncertainty in the data.[7] Finally, the guidelines stress multiple endpoint evaluation from toxicity tests. In an acute toxicity study, for example, it is now recommended that tested animals be examined for subchronic effects, behavioral manifestations, and the identification of target organs, as a means of enhancing the utility of the data derived from animal toxicity tests.

A policy change which may have the effect of reducing the number of animals used for toxicity testing under the requirements of TSCA was the

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[7] Irwin Baumel, personal communication, Office of Toxic Substances.

recent "Change in Test Standards Policy and Test Rule Development Process."[8] This constituted a change in approach to providing test standards for TSCA Sec. 4 test rules. The new approach involves issuing generic test methodology "guidelines" rather than generic test methodology "requirements." Sponsors are allowed to select test protocols listed in TSCA, OECD, or FIFRA guidelines or to submit test protocols of their own, which EPA must approve. Increased flexibility in the performance of toxicity tests is the aim of this change. This should "allow for scientific innovation and encourage the development of more sophisticated and scientifically advanced testing methodologies."[9] Another measure that potentially affects animal usage is the regulatory rule, "Toxic Substances Control Act Data Reimbursement."[10] This rule provides for EPA negotiation of reimbursement for manufacturers or processors of chemicals that perform required testing, from the manufacturers or processors who have been exempted from testing the chemical under Section 4(c) of TSCA. This provision is designed to prevent duplicative testing, and thus reduce unnecessary animal testing. The Agency may also use data submitted under Section 8(e) of TSCA to predict the potential hazard of similar or chemically related substances submitted for premanufacture notice (PMN). This section requires the manufacturer or processor to provide to the Agency any information it obtains on the toxicity of a chemical.

OTS can endorse the use of alternative toxicity tests only when their scientific basis is sound and allows confidence in the data. Human health and protection of the environment are the overriding concerns

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[8] Environmental Protection Agency. 1982. Fed. Regis. 47: 13012-13014.

[9] Ibid., p. 13013.

[10] Ibid., p. 24348.

in toxicity testing. The scientific integrity of toxicity testing cannot be compromised solely to improve animal welfare. Nevertheless, OTS sees a significant role for alternative tests. Inclusion of results from alternative tests with PMN submissions is encouraged, as they enhance the assessment of potential toxicity of new chemicals.

A formal battery of short-term alternative tests as part of the PMN process is, however, viewed with some skepticism within OTS. The present methodologies are considered inadequate to design a static set of tests that provide useful toxicity data for all chemicals. The MPD of the OECD is a test battery employed by some European nations for new toxic chemicals. Ironically, the impetus for its development derived in part from the passage of TSCA in 1976. Some have suggested that introduction of such a test battery in the United States would serve as a negative economic incentive and reduce innovation in chemical research. Alternatively, the MPD may well reduce trade barriers by establishing international standards for toxicity testing regimens. A rapid and reliable toxicity screening program may also help innovation by eliminating more unproductive efforts than good prospects in chemical research.

The OTS has designed a "Retrospective Study of PMN Hazard Predictions," which will soon be undertaken. The study will examine the validity of OTS's use of structure activity relationship (SAR) analyses in the assessment of the potential hazards posed by PMN chemicals submitted to EPA under TSCA.[11] In brief, a sample of 100 chemicals will be selected from the PMN current inventory of over 4000 that have been screened by OTS. They will then be subjected to a battery of toxicity tests, and the results

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[11] SAR analysis attempts to predict the likelihood of toxic effect by comparing the chemical structure of a compound to chemically related compounds of known toxic potential.

compared to the previous predictions of OTS's structure activity team. The test battery will include three short-term mutagenicity assays (Ames Salmonella/microsome test, in vitro sister chromatid exchange assay in Chinese hamster ovary cells, and an in vitro mutation assay in L5178Y mouse lymphoma cells), two general toxicity assays (an acute oral toxicity test in the mouse, and a 14-day or perhaps 28-day repeated dose oral toxicity test in the mouse), and a dermal sensitization assay (the Buehler or "closed patch" test in the guinea pig). This retrospective study will evaluate the effectiveness of EPA's screening protocols, and will certainly have an impact upon any decision to require a short-term test battery for PMN submissions. It will also improve the data base for the performance of SAR analyses and should lead to greater reliability in these analyses. For these reasons, the study is an extremely important effort within EPA to improve the toxicity assessment process.

The OTS has supported an extramural research program on the use of biological markers for carcinogenicity. The establishment of a correlation between the presence of such markers at an early stage and the carcinogenicity of a compound could lead to a large reduction in the number of animals used in lifetime bioassay carcinogenicity studies.

(b) OPP. FIFRA requires the registration of all pesticides distributed in the United States, and establishes the authority of the Administrator to require data in support of the registration. The Data Requirements for Pesticides Registration (40 CFR Part 158), recently published as a Final Rule in the Federal Register,[12] specifies the data and information that must be submitted to EPA to support the registration of each pesticide. Test standards, guidelines on evaluation and reporting

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[12] Environmental Protection Agency. 1984. Fed. Regis. 49: 42856-42905.

of the data, and examples of test protocols are provided in the various subdivisions of the "Pesticide Assessment Guidelines."[13] Based upon a determination of acute toxicity and environmental hazard, pesticides are classified for "general use," meaning any consumer may use them, or for "restricted use" by certified users only. The OPP requires acute toxicity data for oral, dermal, and inhalation effects, and eye and skin irritancy tests. Acute lethality data are required, although a precise LD_{50} is not necessary.

The OPP has the authority to request long-term toxicity data for pesticides that are suspected to remain as residues in foods, or for pesticides which may present other types of chronic exposure. Over the past five years, an increasing number of animals have been used in chronic studies which are performed to comply with the registration and labeling requirements of FIFRA.[14] Test guidelines for chronic studies now recommend 50 animals per sex per dose, with three different dose levels in two species. This has resulted from heightened public concern about the risks of human exposure to pesticides. Although this policy may appear to lead to increased utilization of animals, the adequate testing of pesticides prior to their release for general use may prevent incidents of large accidental field kills of fish and other wildlife, which may number to 10,000 birds and mammals or one million fish.

With respect to animal welfare considerations, OPP concurs with OTS's formulation of test guidelines, and where statutory requirements allow, the new OTS acute toxicity guidelines will be adopted by OPP.

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13 Environmental Protection Agency, Office of Pesticide Programs. 1982. Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals. NTIS-PB83-153916.

14 William Burnam, personal communication, Office of Pesticides Programs.

Short-term in vitro tests presently comprise a major part of the test requirements for pesticides. For example, a battery of tests for the assessment of mutagenicity is required, with considerable flexibility afforded the manufacturer in the choice of specific tests.[15] The design of the battery depends on the nature of the test substance -- it should be able to detect point mutations, structural chromosomal aberrations, and other genotoxic effects. Among the tests that may be included are a variety of in vitro or other alternative tests such as gene mutation tests in microorganisms, the sex-linked recessive lethal test in Drosophila, cytogenetic analysis, cell transformation assays, and DNA repair assays.

Several aspects of the Good Laboratory Practices Standards that have been instituted for TSCA and FIFRA work to reduce unnecessary and wasteful animal testing.[16] Improvement in the quality of the data submitted to EPA reduces the need for repeated testing and lessens duplicative animal tests. An Interagency Agreement between FDA and EPA provides for a coordinated quality assurance program for these agencies' toxicity testing activities. FDA and EPA perform joint data audits and inspections of test facilities. These joint audits ensure that data from toxicity tests are documented and acceptable to the regulatory agencies. In addition, EPA's GLP Standards incorporate guidelines for the proper care and handling of laboratory animals.

(c) ORD. The purpose of the environmental and health effects related research conducted by ORD is to provide information to enable EPA to make better estimates of morbidity and mortality for a given environ-

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15 40 CFR Part 158.135

16 40 CFR Part 160; 40 CFR Part 772 (Subpart B)

mental exposure to toxicants. Therefore, the research conducted can be described in three functional categories:[17]

1. Dose-Response Research -- Studies which directly measure the health effects of specific toxicants. Animal, human, in vitro and in vivo studies for a large number of endpoints and toxicants are conducted.

2. Test Methods Development Research -- Studies to develop improved means of conducting dose-response research. The results of the test methods development research are used both by ORD's own researchers and other research organizations to conduct dose-response research and testing. This includes alternative test methods development.

3. Risk Estimation Methods Development Research -- Studies to develop improved means of making the extrapolations from mouse to man and from high experimental dose levels to lower environmental exposure levels. The results of the risk estimation methods development research are used by other offices to perform health risk estimates as part of the regulatory process.

Food and Drug Administration (FDA)

The FDA is responsible under the Federal Food, Drug, and Cosmetic Act and the Public Health Service Act for assuring human safety and for the protection of animals from the harmful effects of chemicals.[18] In recent years, the FDA has used short-term in vitro tests for several purposes: to set priorities for the selection of chemicals for further testing, to aid in the evaluation of equivocal data from rodent bioassays, and in the determination of mechanisms of action of toxic chemicals. For example, approximately 700 chemical food ingredients known as GRAS (generally recognized as safe) were screened for mutagenicity using short-term in vitro assays, as an assurance that these traditional food ingredients

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[17] Robert Dixon, Office of Research and Development, personal communication.

[18] Environmental Protection Agency, Office of Pesticides and Toxic Substances. 1983. Federal Activities in Toxic Substances. EPA-560/TIIS-83-007.

were not carcinogenic.[19] Very few substances on the GRAS list proved to be genetically active in the in vitro tests, providing assurance that they were indeed safe. Another example is the "Threshold Assessment Guideline,"[20] which provides for short-term in vitro tests to help decide which drugs administered to food-producing animals pose a risk of carcinogenicity. Flamm and Dunkel[21] stated that "short-term tests are used in other areas as well, and are likely to experience even greater use in the future in terms of making decisions about substances that are already on the market, as well as those for which approval is being sought."

A November 1983 Acute Studies Workshop sponsored by the FDA produced position statements on the use of the LD_{50} test from several FDA divisions. A spokesman from the Bureau of Foods stated that his division has no specific testing requirements for cosmetics, and that range-finding tests are more appropriate than the LD_{50} for food and color additives. The Bureau has published guidelines for test procedures that discourage the use of the LD_{50}.[22]

The National Center for Drugs and Biologics of the FDA requires only a general safety test for biologics. The classical LD_{50} is not mandated by the Public Health Service Act. At the Acute Studies Workshop, a spokesman for the Center said that no suitable alternative methods to animal tests exist at present for drugs (which fall under the Food,

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19 Flamm, W. G., and Dunkel, V. C. 1983. Impact of short-term tests on regulatory action. Annals N. Y. Acad. Sci. 406: 395-397.

20 Food and Drug Administration. 1982. Chemical compounds in food-producing animals: availability of criteria for guidelines. Fed. Regis. 47: 4972-4977.

21 Ibid.

22 Food and Drug Administration. 1983. Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food. NTIS PB-83-170-696.

Drug, and Cosmetic Act), but that the intent is to use the smallest possible number of animals. For most new drugs, an estimate of the LD_{50} value is all that is required. The LD_{50} is specifically required for batch testing of a few antitumor drugs, and some regulations may possibly mislead companies into believing the LD_{50} is required for all drugs.[23] The FDA is considering eliminating this limited requirement and is rewriting its other regulations to clarify its position on the LD_{50}.

The Bureau of Veterinary Medicine regulates drugs used to treat animals, and chemicals used as additives in animal feed. It has no requirement for the LD_{50}, and emphasizes low-dose chronic testing of any substance that can become a component of human food, because it remains as a residue in animals used as food.

Department of Transportation (DOT)

DOT regulates the shipment of hazardous materials in commerce under the Hazardous Materials Transportation Act. The Office of Hazardous Materials Regulation requires some toxicity testing and participated in the FDA workship on acute studies in November 1983. Test conditions, types of animals, and minimum number of animals to be used are specified in its hazardous materials regulations.[24] DOT classifies hazardous materials as either Class A or Class B poisons. Class A poisons are poisonous gases or volatile liquids, for which exposure to the vapors is dangerous at very low levels. DOT does not require LD_{50} data for Class A poisons. Class B poisons are liquids or solids known to be toxic to humans or animals, and which may therefore create a potential hazard during transport. For hazard Class B poisons, acute oral, inhalation, and skin

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23 Norman, C. 1984. Science 225: 1005.

24 49 CFR 171-179.

absorption tests are required, but not specifically an LD_{50}. Some acute lethality tests are, however, performed to comply with DOT regulations.

Consumer Product Safety Commission (CPSC)

The CPSC regulates toxic chemicals under the Federal Hazardous Substances Act (FHSA). Toxicity data are required for labeling purposes and regulations specify the test conditions, the animal, and the minimum number of animals required for testing.[25] The CPSC recently published a statement of policy on animal testing which "is intended to reduce the number of animals tested to determine hazards associated with household products and to reduce any pain that might be associated with such testing."[26] The policy statement further encourages the use of existing alternatives to animal tests, including "prior human experience, literature sources which record prior animal testing or limited human tests, and expert opinion."

When animal testing is found to be necessary, the CPSC has implemented the following procedures: (i) Acute toxicity is determined by a limit test rather than a classical LD_{50} test. This reduces the number of animals used from 80-100 to 10-20, and is acceptable because the FHSA and CPSC regulations do not require a precise LD_{50}. (ii) Eye irritancy testing is not performed if a product is known to be a primary skin irritant, since the latter are usually also eye irritants. When eye irritancy testing is required the animals are treated with an opthalmic anesthetic to reduce their pain and suffering. (iii) The use of stocks for restraint of animals during skin irritation testing has been eliminated. This allows free

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[25] 16 CFR 1500-1512.

[26] Consumer Product Safety Commission. 1984. Fed. Regis. 49: 22522-22523.

mobility and access to food and water, and has eliminated the stress and dehydration previously encountered. The CPSC encourages manufacturers of products subject to the FHSA to adopt similar procedures.

B. The Animal Welfare Community

The animal welfare community consists of diverse groups, including antivivisectionist organizations, humane groups, and animal rights advocates. There is a spectrum of positions within the animal welfare community. Some groups favor complete abolition of all animal testing and research based on social and ethical concerns. Other groups acknowledge a necessity for animal research and testing, but propose a reduction of the numbers and suffering of the animals used in toxicity testing. Numerous animal groups have coordinated their efforts to achieve elimination of specific tests which they find particularly offensive, such as the Draize Eye Irritancy Test and LD_{50} Acute Toxicity Test. The following represents a very small sample of the numerous organizations devoted to protecting the welfare of animals.

Ethical Issues and Animal Rights

Ethical arguments against the use of animals in testing and research have been based on assertions of basic rights that animals possess and which are denied in the conduct of animal experimentation. How these rights are defined depends on philosophical perspective, and varies from a complete right to life to a less sweeping right to freedom from pain. There is general agreement, though, that the use of animals in painful research and testing or for perceived trivial purposes is immoral.

Dr. Thomas Regan, Professor of Philosophy and Religion at North Carolina State University, articulated the position of those who define

animal rights very broadly at an NIH-sponsored meeting on "Trends in Bioassay Methodology" in 1981.[26] He disagreed with scientists who speak of animals as "models" or "tools," seeing this as a "symptom of our vanity or our insecurity to suppose that they are here for us as models, as tools for us, a gift of a thoughtful deity or a beneficent evolutionary scheme." He argued that animals are like humans in morally relevant ways. "Their life has a value to them independent of their utility to us. They can be harmed, not only by being made to suffer, which is an important consideration, but by being denied various opportunities." He cited denial of the liberty to move around and the premature ending of their lives as examples of a notion of harm that is "more extensive than the notion of suffering."

The central point of his argument is that one cannot justify using animals for research purposes merely because it benefits humans. The benefits that derive from harming others is never an adequate justification of that harm. He also noted that "it is a pernicious prejudice to discriminate against human beings on the basis of their sex or on the basis of their race." He argued that "if we accept this -- if we get to the point where we say biological differences don't mark moral boundaries -- then we can't rationally hold that belonging to a particular biological species (Homo sapiens) makes us morally superior. No mere biological difference, even species membership, marks any moral boundary."

The term "speciesism" has been used by Peter Singer[27] to describe "a

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[26] Regan, T. 1981. In "Trends in Bioassay Methodology: In Vivo, In Vitro, and Mathematical Approaches," pp. 115-119. Department of Health and Human Services, Public Health Service, NIH Pub. #82-2382.

[27] Singer, Peter. 1975. "Animal Liberation: A New Ethics for our Treatment of Animals." New York: New York Review.

prejudice or attitude of bias toward the interests of members of one's own species and against those of members of other species," a prejudice "immoral and indefensible in the same way that discrimination on the basis of race is immoral and indefensible." Singer argues that the capacity for suffering and/or enjoyment or happiness is the essential characteristic that gives a being the right to equal consideration. The limit of sentience is the only defensible boundary of concern for the interests of others. In his view, this boundary cannot be drawn so as to include only humans.

Fund for the Replacement of Animals in Medical Experiments (FRAME)

FRAME was established in England in 1969 as an independent charitable institution to promote the concept of alternatives to animal testing; it concentrates its efforts on scientific research organizations. FRAME does not consider itself an antivivisection organization and does not oppose medical and veterinary research on animals nor animal testing of drugs and chemicals when essential for continued progress against disease and for the protection of human safety. FRAME supports research on alternatives in England and in the United States, and has been very influential in the animal alternatives debate in both countries. The FRAME Toxicity Committee Report[28] of November 1982 concluded:

> Although animal models are of limited value in predicting toxic hazards for man, there is as yet little evidence that it will be possible in the foreseeable future to dispense entirely with live animal testing... Total abolition of the need for animal experimentation is a longer term goal, since, with few exceptions, alternative approaches and methodologies are not yet developed to the point where they could conceivably be considered as adequate total replacements.

The Committee recommended maximum scientific use of every animal

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28 In "Animals and Alternatives in Toxicity Testing" (eds. Balls, Riddell, and Worden), pp. 501-540. New York: Academic Press, 1983.

that has to be used in any form of toxicity testing, and stated that formal LD_{50} tests should not be required for most pharmaceuticals and many other chemicals and "should be replaced by more meaningful acute toxicity studies involving the use of fewer animals."

Coalition to Abolish the LD_{50} and Draize Tests

A large number of animal welfare organizations are affiliated with this coalition, whose primary goal is the abolition of the LD_{50} Acute Toxicity Test. With respect to the LD_{50}, this group has an uncompromising point of view: that the LD_{50} is archaic and should be abolished. The Coalition asserts that this test, because of its focus on lethality as the endpoint, provides no information about affected organs, symptoms, or long-term effects. It urges the substitution of the Approximate Lethal Dose (ALD) or limit test. The Coalition asserts there is consensus in the scientific community that the LD_{50} is useless and should be replaced by alternative testing methods.[29]

The Coalition's rhetoric has in general been more aggressive than its negotiating style. It has been willing to compromise to promote dialog between scientists and animal activists, and has recognized that progress will be gradual and dependent upon further advances in the science of toxicology. For example, in 1980-1981 the Coalition conducted a campaign against the Draize Eye Irritancy Test. It played a major role in persuading the Revlon Corporation, the primary target of the campaign, to award a $750,000 grant to Rockefeller University for a research program to investigate alternatives to the Draize test. This agreement ended the

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[29] Open letter from Henry Spira, Director, Coalition to Abolish the LD_{50}, September 15, 1983.

unfavorable publicity that the cosmetic industry had attracted during the campaign, elevated the awareness of the scientific community to the issue of animal alternatives, and initiated changes in the use of the Draize test in industry, such as increased use of anesthesia.

United Action for Animals

This animal welfare organization was founded specifically to promote alternative methods of testing and research, and has focused on methods which can ultimately be "replacements" for animal techniques. It drafted the Research Modernization Act (H. R. 556), which was first introduced in 1980. If passed, 30-50% of all federal funds for research and testing that use live animals would have been diverted to create a National Center for Alternative Research.[30] This bill also would have eliminated or minimized duplication of experiments with live animals.

The Humane Society of the United States

The Humane Society promotes the "3 Rs" interpretation of alternative testing as the soundest approach to accomplishing a reduction in the use of animals for toxicity testing. They recognize that "reduction" and "refinement" are interim steps towards the ideal of total replacement of animal tests. As its principal effort in the area of toxicity testing, the Society has focused on the elimination of the LD_{50} and Draize tests. In a recent Humane Society position paper, their technical and ethical criticisms of the LD_{50} are presented.[31] The LD_{50} is

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[30] Zola, J. C., Sechzer, J. A., Sieber, J. E., Griffin, A. 1984. Animal Experimentation: Issues for the 1980s. Science, Technology, and Human Values 9: 40-50.

[31] The Humane Society of the U. S. 1984. Fact Sheet: Classical LD_{50} Acute Toxicity Test. Washington, DC.

described as unnecessary for protecting human health. The Society asserts that "biological differences between animals and humans severely limit the usefulness of LD_{50} data in safety testing." It further criticizes the LD_{50} as having little use for medical diagnosis and treatment. (For example, little or no information is yielded from the LD_{50} about poisonous or lethal doses for humans, symptoms of overdoses, long-term effects, etc.) The LD_{50} is also seen to be unreliable because of experimental variables, and test results are consequently often invalid. The Society's ethical objections to the LD_{50} can be summarized: (i) It causes needless pain and suffering, (ii) it wastes animal life, and (iii) alternatives are available, such as the Approximate Lethal Dose, the limit test, computer-based predictive systems, and cell culture methods. The Society suggests that the primary reason for the continued use of the LD_{50} is "bureaucratic inertia", and that when regulatory requirements or guidelines recommending its use are eliminated, the LD_{50} will be soon abandoned by the scientific community.

With respect to the Draize test, similar objections are raised by the Humane Society.[32] The technical flaws of the Draize test identified by the Society include: (i) the results of eye-irritancy testing on rabbits are of questionable applicability to the human situation, (ii) the test lacks fine discrimination, and (iii) test results are difficult to reproduce and, as a result, unreliable. The Society cites the great suffering of the subject animals and the availability of non-animal alternatives as ethical objections to the Draize test.

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[32] The Humane Society of the U. S. 1985. Fact Sheet: Draize Acute Eye-Irritancy Test. Washington, DC.

C. The Chemical, Pharmaceutical, and Cosmetic Industries

Since the manufacturers of chemicals, drugs, and cosmetics perform most of the toxicity testing required by law, there are clear economic incentives for them to replace whole-animal tests with less expensive short-term in vitro methods. However, there are reasons other than compliance with the law for these companies to test their products prior to marketing, which may lead to increased animal testing. First, manufacturers want to develop products that consumers will like and buy again. Second, manufacturers need to establish safety substantiation records to protect themselves against product liability suits. Evidence of a violation of a health or safety regulation is tantamount to establishing liability if the product can be shown to have caused an injury. Therefore, federal "guidelines" tend to become de facto requirements for certain kinds of testing, regardless of the good intentions of regulatory agencies to maintain flexibility. Third, some companies may be hesitant to employ alternative methods because they wish to avoid risking rejection of their products by regulatory agencies. Also, many products come under the jurisdiction of several agencies, and the manufacturer must therefore comply with the strictest regulations. This kind of risk-averse behavior is understandable when the massive investment necessary to market a product is considered. These factors explain the concern about inconsistencies and ambiguities in regulations which may cause unnecessary, excessive, or dulicative animal testing. The positions on animal testing of several trade associations follow.

Pharmaceutical Manufacturers Association (PMA)

PMA's position on the role of the LD_{50} in drug safety evaluation is

enunciated as follows:[33]

> Although there is no adequate substitute for acute toxicity studies involving the use of laboratory animals, a review of the utility of the precise LD_{50} test reveals that neither the toxicologist nor the clinical pharmacologist needs a precise LD_{50} value. In consideration of the final use of the data, carefully controlled and professionally performed acute experiments can be conducted which require fewer animals and yet are more meaningful and relevant to the introduction of therapeutic agents than the LD_{50} test. Regulatory requirements should accommodate this position.

Chemical Manufacturers Association (CMA)

The CMA represents over 200 companies, which produce more than 90% of the chemicals manufactured in this country. At the Acute Studies Workshop sponsored by the FDA in November 1983, a CMA representative held that traditional tests have provided a great deal of useful information to the industry, and that there are no readily available alternatives to the LD_{50}. However, CMA sponsors a number of activities which effectively reduce the number of animals used in toxicity testing. For example, CMA has developed a comprehensive system for cooperative testing among its member companies. Any member may suggest a cooperative testing effort for a particular compound. CMA then informs other manufacturers of the request, and if there is sufficient interest, CMA decides the feasibility of the proposed project and is responsible for its administration. Joint testing acts to reduce the number of animals used in testing, and at the same time saves money for the companies involved. The agreement allows for some confidential information such as production capacity and sales volume to be kept secret by participating companies.

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[33] U. S. Food and Drug Administration, Office of Science Coordination. Final Report on Acute Studies Workshop, Nov. 9, 1983, p. 6.

Cosmetic, Toiletry, and Fragrance Association (CTFA)

The cosmetics industry has been a special target of animal welfare advocates. They have been especially vocal in opposition to two tests commonly performed by companies in this industry -- the LD_{50} acute toxicity and Draize eye irritancy tests. The LD_{50} test measures the dose (in a single administration) that kills 50% of a test group of animals. The Draize test is designed to provide information about eye irritancy caused by test materials (chiefly ingredients in cosmetic products) by exposing the eyes of rabbits to the materials. A well-organized campaign against the Draize eye irritancy test attracted public attention to the animal testing practices of cosmetic manufacturers. Consequently, CTFA has been active in the alternative testing debate. Most prominent among its activities in this area was an award of one million dollars to the Johns Hopkins Center for Alternatives to Animal Testing. The following statement made by a CTFA representative during a symposium on animal alternatives summarizes their position.[34]

> Both as a matter of good business practice and because of legal requirements, cosmetic manufacturers are concerned to market safe products. Unfortunately, safety substantiation sometimes requires the use of animal testing. Cosmetic manufacturers would like to avoid such use of animals in the development of their products. Cosmetic companies share their customers' concerns for the humane treatment of animals. Our companies would like to reduce to the irreducible minimum the use of animals in the testing of cosmetics. Nevertheless, at present, animal testing is sometimes the only acceptable means for assuring the safety of consumer products such as cosmetics.

With respect to the LD_{50}, CTFA's Board of Directors released the following statement: "All manufacturers of cosmetic, toiletry, and fragrance

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[34] McNamara, S. H. 1983. Legal Requirements for Safety Testing of Cosmetics. In "Product Safety Evaluation," (ed. A. M. Goldberg). New York: Mary Ann Liebert.

products are encouraged not to use the LD_{50} test except in those cases where this specific test is essential to provide appropriate assurance for consumer safety."[35]

D. The Not-for-Profit Research Community

Within this community, there is widely varying opinion regarding the potential of alternative tests. Most members of the research community express a need for continued use of animals, at least in some areas of research and testing, for the foreseeable future. The testing of drugs, for example, is often cited as not amenable to alternative techniques, because pharmacological activity may be systemic and therefore impossible to identify in simple in vitro systems. There is, however, considerable disagreement about the extent to which alternative methods may be appropriate for particular applications, and there is optimism that in the area of reduction and refinement of animal testing, progress is possible.

National Institutes of Health (NIH)

Dr. James Wyngaarden, Director of NIH, in a letter to Sen. William Proxmire,[36] described the activities supported by NIH for the reduction of animal use in toxicity testing. Examples that he cited included the "International Program to Evaluate Short-Term Tests for Carcinogenicity" and efforts to apply methods that use a limited number of animals. The latter is illustrated by the benzidine carcinogenesis testing protocol, results from which will allow generalization to a whole class of chemicals and eliminate the need to test the chemicals in this class individually.

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[35] U.S. FDA, Final Report on Acute Studies Workshop, Nov. 9, 1983, p. 5.

[36] In Touch... New Methods in Toxicology. 1982. Vol. 1(2): 7.

In vitro tests are presently being used to screen chemicals for mutagenic activity in order to establish priorities for 2-year animal studies. The following practical results from NIH's efforts to reduce animal testing were outlined by Dr. Wyngaarden: reduction in reliance on the LD_{50} for acute testing data by the National Toxicology Program (NTP); adoption by the NTP of in vitro tests for genotoxicity; development of various cell and organ culture systems for testing teratogens at the National Institute of Environmental Health Science.

Dr. Wyngaarden expressed the opinion that in vitro systems will not supplant animal tests in the near future. They are more likely to be useful as screens, which may restrict the use of animals to the confirmation of likely human risk. This will certainly reduce the number of animals used in testing. He stated further:[37]

> Animal research has contributed directly to a trend toward decreasing use of animals by leading researchers to non-animal systems where molecular questions can be answered... Progress in biomedical research requires that we work within systems which will yield useful information. As in the area of biological testing, we do not believe that animals can be totally eliminated from biomedical research - or, if we remain committed to the fullest benefit from that research, that they should be.

The NIH has contracted with the National Academy of Sciences for an evaluation of nonmammalian models in biomedical research. This is an investigation of the opportunities for and limitations of the use of lower organisms, in vitro methods, and nonbiological systems as models for biomedical research. The conclusions of this study will certainly have important implications for animal toxicity testing. The report was released in April 1985.

The National Cancer Institute (NCI) has enumerated its activities

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[37] Ibid.

encouraging the reduction of animal usage in research.[38] Programs investigating the replacement of testing in mice with in vitro tests include: A $3.2 million evaluation of the clonogenic assay; $500,000 per year for correlation of in vivo and in vitro screening models; $170,000 per year to perform biochemical and in vitro tests as adjuncts to animal tests for screening chemical compounds; assistance projects for in vitro methods such as $500,000 per year to a Request for Applications for grants in the area of multi-drug resistant human tumor cell lines, and $425,000 to screening models for cancer chemotherapy drug development. The Division of Cancer Cause and Prevention spends $3.1 million per year on cell culture, bacterial, and other non-animal systems for testing carcinogens, mutagens, and cancer promoters, and for mechanistic studies. The Clinical Pharmacology Branch reported at a 1983 FDA Acute Studies Workshop that it focuses on the maximum tolerated dose (MTD) rather than the LD_{50} in tests for anti-cancer drugs. One tenth of the LD_{10} (10% lethality) in mice is used to establish the starting dose for clinical trials.[39]

National Center for Toxicological Research (NCTR)

This organization is the primary research and testing division of FDA. Dr. Ronald Hart, Director of NCTR, responding to a letter from Henry Spira of the Coalition to Abolish the LD_{50}, stated that the NCTR is developing alternative tests and is trying to reduce the trauma to animals in present testing protocols.[40] Examples cited were: administration of volatile or

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[38] Memorandum, April 12, 1983, from J. Paul Van Nevel, Assoc. Dir. Cancer Communications, NCI, to James Willett, Division of Research Resources, NIH.

[39] U. S. FDA. Final Report on Acute Studies Workshop, Nov. 9, 1983, p. 4.

[40] In Touch... New Methods in Toxicology. 1982. Vol. 1(2): 7.

unstable chemicals by microencapsulation in food rather than by gavage; statistical methods that allow determination of toxicity values with fewer animals; in vitro methods for teratogenicity; and cell and organ culture systems. To quote Dr. Hart, "in the foreseeable future we will not be able to entirely eliminate the use of animals in the field of toxicology, but as we understand more about the mechanisms upon which toxicity is based, we will be able to better mimic these systems outside of the animal."

National Toxicology Program (NTP)

The NTP is a Department of Health and Human Services cooperative effort consisting of the relevant toxicology activities of the National Institute of Environmental Health Sciences (NIEHS) of NIH, the FDA's NCTR, and the National Institute for Occupational Safety and Health (NIOSH) of the Center for Disease Control (CDC). The major part of NTP's budget is devoted to toxicity testing, with a significant portion spent on methods development and validation. The Program represents "a leading effort in the world aimed at developing better, faster, and less expensive methods for determining whether chemicals may be hazardous."[41]

Through coordination of the toxicology research and testing activities of its member agencies, NTP improves testing of toxic chemicals and provides better information for risk estimation. NTP communicates actively with other federal agencies and the private sector, and thus helps to reduce duplication of efforts and avoid unnecessary testing. As part of its function, NTP annually publishes a Review of Current DHHS, DOE, and EPA Research Related to Toxicology. The EPA research reported in this NTP

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[41] National Toxicology Program Annual Plan, Fiscal Year 1984, p. 7. U.S. Department of Health and Human Services, Public Health Service.

document is that conducted by the Office of Health Research in ORD.

EPA and NTP cooperate in a number of ways. For example, the NTP/EPA Clearinghouse on Phthlates is an effort to coordinate information from research on the toxic effects of these compounds. NTP also tests chemicals that have been identified by EPA in priority hazardous waste sites, under the Comprehensive Environmental Response, Compensation, and Liability Act (Superfund). Scientists from EPA were involved in the formulation of the Benzidine Dye Initiative, which is a study of the metabolism and toxicology of a class of dyes derived from benzidine.

Representatives from EPA and a number of other government agencies serve on the Chemical Evaluation Committee (CEC) of the NTP. The Committee evaluates chemicals that have been nominated for testing by federal or state agencies, industry, labor, or the public, and recommends the types of testing to be performed. Following opportunities for public comment and peer review of the CEC's recommendations, the NTP Executive Committee makes final decisions on whether to test nominated chemicals.

The National Society for Medical Research (NSMR)

The NSMR is a professional association of medical researchers. In a position paper on animal research,[42] the Society indicates that data from in vitro tests will always be imperfect because of the complex and interrelated actions by and on the chemical in the intact animal, and therefore in vitro tests are inadequate for toxicity testing. They argue that the possibility of false negatives, due to the simplicity of in vitro testing models, makes it unwise and scientifically unsound to substitute in vitro tests for animal test methods. In vitro tests may serve to reduce

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[42] Adjunct Methods of Testing and Research: An Open Letter. 1982. Published by the National Society for Medical Research.

animal testing in certain types of simple toxicology testing, for example in testing for irritancy, but beyond this, there is difficulty. In vivo and in vitro testing methods can be complementary; however, the NMSR believes that the use of in vitro methods alone erroneously assumes a more complete state of knowledge of structure and function of living beings than scientists now possess. The NSMR believes "adjuncts" rather than "alternatives" is a more accurate description of in vitro tests, as used by "individuals who believe that it is possible to use laboratory tests to replace animals in research and testing to improve and protect human and animal life."[43]

American Psychological Association (APA)

APA has advocated a "balanced and deliberative approach" to the humane treatment of laboratory animals, in which there must be a sound basis for rejecting current research methods and no risk of jeopardizing productive research.[44] The absence of alternatives reflects the "necessarily slow process of developing such alternatives." APA supports the NAS study provision of the NIH reauthorization bill which will improve the data base for policy decisions relating to the use of animals in research. "Alternative methods for studying behavior for the most part are not feasible. Studying behavior requires studying live animals."

The Johns Hopkins Center for Alternatives to Animal Testing

The Center was established with a $1 million grant from the Cosmetic, Toiletry, and Fragrance Association. The role of the Center is to conduct

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[43] Ibid.

[44] Position Statement on Animal Research Legislation. Published by the APA Office of National Policy Studies on Animal Research Legislation, Dec. 1983.

an "independent, impartial search for alternatives to animal testing" with a focus on basic research to arrive at a better understanding of the mechanisms of toxicity.[45] The Center's Director, Dr. Alan Goldberg, has cautioned that "As a practical matter there will be a need to rely on animal tests for some time to come to protect the public, and to advance the frontiers of medical knowledge."

Scientists' Center for Animal Welfare

This group was established in 1978 in an attempt to persuade the scientific community to adopt a balanced and nondefensive attitude regarding animal welfare. The following excerpts describe the objectives and functions of the Center.[46]

> The major objective is to help sensitize scientists to the issues involved in the humane treatment of animals. It stands on the general principle that all matters of public concern should be freely discussed, and that scientists themselves should take the initiative in establishing and maintaining a high credibility and accountability in matters of public conscience.
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> The functions of the Center are to foster humane stewardship of animals by educating scientists and the public about animal welfare; to promote intelligent and humane decisions in establishing public policy; to collect and exchange scientific information relevant to animal welfare; and to encourage universities and professional schools to offer courses on the ethical aspects of our interrelationships with animals and on the technical skills involved in handling animals.

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[45] Goldberg, A. M. 1983. The Johns Hopkins Center for Alternatives to Animal Testing. In "Product Safety Evaluation" (ed. A. M. Goldberg), pp. 3-14. New York: Mary Ann Liebert.

[46] Dodds, J. W., and B. F. Orlans, eds. 1982. In the Preface to "Scientific Perspectives in Animal Welfare." New York: Academic Press.

III. REGULATORY POLICY ISSUES

Regulatory policy issues emerge as changes occur in the use of animals in toxicity testing and research. The material assembled in the previous section provides necessary background for use in developing solutions to the following problems.

1. ISSUE: Agency-wide criteria for the evaluation and adoption of alternative toxicity testing methods

Scientific criteria for establishing the usefulness of an alternative toxicity test should be based on (1) ability to demonstrate a toxic effect, (2) sensitivity, (3) reproduciblity, (4) extent of application, (5) ease of standardization, and (6) cost. A new test should be at least as good as existing toxicity tests to ensure that human and environmental safety are not compromised. It is important that a formal process for validation of alternative methods be established that applies specific criteria for measuring a test's effectiveness.

2. ISSUE: Periodic review of toxicity testing methods.

Upon validation, an alternative method may be incorporated into testing protocols utilized for regulatory purposes. It is important that both new test methods and currently used methods be periodically reviewed to assess their reliability in predicting human or environmental hazards. As a new method is used to test chemicals, more reliable information about its ability to assess toxicity will be obtained. Some refinements in the methodology may be needed, or perhaps replacement with a method that more accurately predicts human and environmental health effects. Likewise, new technology may make older test methods

obsolete, either because of technical superiority or lower cost. The periodic review of testing methods should involve a commitment to modify or discard tests that are not cost-effective or do not provide good indications of hazard.

3. ISSUE: Consistency of policy among federal agencies

Each federal regulatory agency operates under different legislative mandates. Toxicity testing practices reflect this diversity of responsibilities. There is, however, consensus in the scientific and regulatory community that precipitous elimination of the use of animals would diminish the scientific quality of toxicity evaluation, and increase the risk to humans and to the environment. At the same time, it is generally held that some reductions, refinements, and, ultimately, replacements of animal tests are possible. This consensus suggests that despite the constraints of each regulatory agency's statutory authority, it may be possible to articulate a general federal policy on alternatives to animal toxicity testing. At a minimum, it should be possible to formulate general guidelines on the development and adoption of alternative methods.

4. ISSUE: The relationship of federal to other governmental regulations and guidelines on the performance of toxicity testing.

The creation of trade barriers in particular industries is possible when international toxicity testing practices vary. For example, as a result of perceived inadequacies in the testing protocols of one country, a product manufactured in that country may not be approved for sale in another. A large country with a significant market for a product can

influence the testing procedures of manufacturers. Manufacturers will naturally seek the largest market possible for their products, and may employ stringent testing protocols to ensure the broadest product approval.

The previously mentioned "Change in Test Standards Policy" (p. 12) allows manufacturers to employ TSCA, FIFRA, or OECD guidelines for test protocol development to comply with TSCA testing requirements. FIFRA testing requirements for pesticide registration are not as flexible, however, nor are many of the testing requirements of other federal agencies. Compliance with both federal and international toxicity testing requirements can therefore be complex and often redundant. Consistent international guidelines on toxicity testing might alleviate some of these problems.

5. ISSUE: Access to data relevant to alternative test development and the dissemination of such information.

Numerous agencies require toxicity testing to carry out their legislative mandates. The existence of a variety of bibliographic computer data bases greatly facilitates the search for information on alternative methods. It is possible to rapidly obtain a comprehensive list of journal articles, conference reports, and research proposals on any subject (see Appendix A). A number of these data bases are devoted to toxicology, and many to biomedical research. However, since the emphasis of most reports is on basic research, more often than not the authors do not discuss the potential applications of their research results to toxicity testing. This problem can be circumvented by carefully designing the search strategy to include a variety of descriptors, so that most of the relevant reports are identified. With practice, and particularly with

the assistance of information specialists, an extensive literature for methods that have potential as alternatives can be surveyed.

The primary method of evaluation of new chemicals for regulation under TSCA is most often SAR analysis. SAR analysis is seriously handicapped by the absence of a large data base containing toxicologic test data, physical chemical property data, and quantitative structure-activity relationship (QSAR) descriptors. Available substructure and nomenclature search systems, such as the SANSS data base on the Chemical Information System (CIS), restrict searchable parameters to chemical names, name fragments, substructural components, molecular formulae, etc. Although these systems are invaluable, they do not contain all of the data needed for SAR analysis. Automated screening to identify analogs of new chemicals using physical chemical properties is therefore not possible. This makes the identitication of appropriate analogs to new chemicals, upon which a subsequent literature search for toxicological data is based, a difficult and laborious process. An effective system for obtaining this information would improve confidence in SAR analysis and, because the majority of decisions requiring further toxicity testing for new chemicals are based on SAR analyses, probably reduce the need for animal toxicity tests. An information system on toxicological methods development would ease access to data and foster the elimination of duplication in testing. Also, a specialized quantitative SAR database would improve the quality of SAR analysis.

6. ISSUE: Identifying alternative testing methods from studies of environmental effects of toxic chemicals.

A great deal of information about toxicity is gained from studies on

the environmental effects of chemicals. Many of the species studied in ecotoxicity research (e.g., fish and invertebrates) may be more acceptable as test species than are mammals. The development of model systems for studying the mechanisms of toxic effects may be achievable in such species. Ecotoxicity studies represent a potentially large and promising source of alternative methods in toxicity testing.

7. ISSUE: The development of incentives for the transfer of technology from the laboratory to practical application.

Technology transfer has been defined as the application of basic research to problems practical in nature.[47] The basic research process of toxicology typically involves devising a model system for use as a human analog, identification of the toxic endpoint from the model, and attempts to understand the biochemical mechanism of the toxic effect. The development of a toxicity test from basic research findings may not necessarily be the intent of the researcher. Incentives which foster development of excellent new alternative toxicity tests would be desirable.

8. ISSUE: Public dialogue about new toxicity testing schemes.

The increase in the number of animals used for toxicity testing over the last several decades is a direct result of public pressure for safer consumer products. Many laws designed to protect human and environmental health were passed requiring the manufacturers of new products to test their products for potential hazard before they reach the market-

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[47] Brusick, D. 1984. From methodology to assay procedure: the transfer of technology. Paper presented at the symposium "In Vitro Toxicology," The Johns Hopkins Center of Alternatives to Animal Testing, Baltimore, MD, Oct. 23-24, 1984.

place.

Questions may persist about the reliability of extrapolating data from alternative tests to human health effects. Unanimity of opinion in the scientific community about the appropriate use of alternatives is unlikely, so public debate over the use of alternative methods in regulation may emerge. As the Agency decides whether or not to approve the use of particular alternative methods as replacements for currently used test methods, an effort to engage public discussion of the basis for these decisions should enable the implementation of testing protocols that ensure human safety and that minimize the pain and distress of animals.

9. ISSUE: The possible passage of legislation that would require changes in toxicity testing procedures.

Legislation has been proposed that would limit the use of animals for toxicity testing and research and would prescribe extensive use of alternative methods (see Appendix B). In addition, the alternative testing debate may affect several other pieces of legislation, including TSCA reauthorization and the research institutions' appropriations. Still other legislation may require a battery of short-term tests to be conducted as part of the PMN submission process of TSCA. There are four possible outcomes with respect to legislative action on alternatives to animal testing: (i) to eliminate animal use gradually; (ii) to eliminate animal use precipitously; (iii) to reform the treatment and reduce the numbers of animals used in research; (iv) or to maintain the status quo.

Although not uniformly favored at present, a "test battery" may be considered in response to the uncertainties of SAR analysis. A required

test battery would serve to allow some risk assessment of all new chemicals based on actual toxicity data. Confidence in the tests included in a test battery would be broadened as test data accumulated. On the other hand, the danger of standardization and rigidity is a major disadvantage of such a test battery. Reduction in flexibility would be unfortunate, considering the immaturity of development of short-term toxicity tests and the rapidity of change in this field. It would therefore be critical to ensure a process for revision and updating of any test battery as the state-of-the-art improves. Also, a required test battery could quite possibly lead to more animals being used, since some of the tests utilized would certainly require the use of animals, and every newly introduced chemical would need to be tested. An as yet unanswered question is whether the information derived from a standard battery of tests would justify the expense of the additional testing.

IV. CASE STUDY: AVAILABLE RESOURCES FOR THE IDENTIFICATION OF ALTERNATIVE TOXICITY TESTING METHODS IN NEUROTOXICITY AND BEHAVIORAL TOXICITY

In order to assess the ease of access to information about alternative techniques in toxicity testing, neurotoxicity and behavioral toxicity were chosen as the subjects for an extensive literature search, employing computer bibliographic data bases supplemented with some manual searching. These areas were selected because they have received less emphasis in the effort to find testing alternatives than mutagenicity, carcinogenicity, and reproductive toxicity testing. Also, since fewer assays exist for neurotoxicity and behavioral toxicity, the search was more manageable to perform as a case study.

As the expression of extremely complex biochemical and physiological interactions, behavior is often highly sensitive to toxic chemicals. On the other hand, many chemicals of known toxicity have no discernible effect on behavior. Clearly, behavioral tests cannot be solely relied on to provide information about toxicity. But it is possible that some behavioral toxicity tests may be better able to detect certain low-level toxic effects than traditional methods, and would therefore be very useful as part of a battery of screening tests. Virtually all behavioral toxicity studies involve the use of live animals. This is not surprising, since "behavior" requires an intact organism. Many of these studies may nevertheless be viewed as alternatives, if they lead to reduction or refinement of animal toxicity tests.

A comprehensive search for data does not, of course, ensure that the quality of the data is acceptable. After the information is collected, the next step must be a review by experts to determine which studies have the greatest potential for the development of alternative tests. Since

the research is not necessarily directed toward the development of alternative tests, some mechanism for encouraging investigators to pursue promising applications to test development should be established.

Appendix A describes the design and performance of the case study, and specifies the various information resources that identify potential alternative methods for assessing behavioral toxicity and neurotoxicity. The opportunities and limitations of the process employed to identify these resources are discussed. A list of journal reports and research proposals that were identified in the case study is provided. No attempt was made to review these studies, other than to verify that they were relevant to the subject of behavioral toxicity and neurotoxicity. The task of reviewing this information must be accomplished by experts in these disciplines.

Table 2

Acronyms Used in the Report

ALD	Approximate Lethal Dose
APA	American Psychological Association
CDC	Center for Disease Control
CEC	Chemical Evaluation Committee
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
CFR	Code of Federal Regulations
CIS	Chemical Information System
CMA	Chemical Manufacturers' Association
CPSC	Consumer Product Safety Commission
CRIS	Current Research Information System
CRISP	Computer Retrieval of Information on Scientific Projects
CSIN	Chemical Substances Information System
CTFA	Cosmetic, Toiletry, and Fragrance Association
DHHS	United States Department of Health and Human Services
DOE	United States Department of Energy
DOT	United States Department of Transportation
EPA	United States Environmental Protection Agency
FDA	Food and Drug Administration
FHSA	Federal Hazardous Substances Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FRAME	Fund for the Replacement of Animals in Medical Experiments
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GRAS	Generally Recognized as Safe
LD_{50}	Lethal Dose 50%

MPD	Minimum Premarket Dataset
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NCTR	National Center for Toxicological Research
NIEHS	National Institute for Environmental Health Sciences
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NSMR	National Society for Medical Research
NTIS	National Technical Information Service
NTP	National Toxicology Program
OECD	Organization for Economic and Cooperative Development
OPP	Office of Pesticides Programs
OPPE	Office of Policy, Planning, and Evaluation
OPTS	Office of Pesticides and Toxic Substances
ORD	Office of Research and Development
OTA	Office of Technology Assessment
OTS	Office of Toxic Substances
PHS	Public Health Service
PMA	Pharmaceutical Manufacturers Association
PMN	Premanufacture Notice
QSAR	Quantitative Structure-Activity Relationship
SAR	Structure-Activity Relationship
SSIE	Smithsonian Science Information Exchange
TSCA	Toxic Substances Control Act
USDA	United States Department of Agriculture

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Studies of Development Using Nonmammalian Models, Committee on Models for Biomedical Research, NAS Workshop, Woods Hole, MA, Aug. 15-17, 1984.

Religious Perspectives on the Use of Animals in Science, American Fund for Alternatives in Animal Research, London, July 25-27, 1984.

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Appendix A

Resources for Accessing Information on Alternative Methods - Neurotoxicity and Behavioral Toxicity

A number of review articles were identified that provide summaries of available neurotoxicity testing methods. Among the most useful was the chapter on neurotoxicity in the recent report of the Fund for the Replacement of Animals in Medical Experiments (FRAME), "Animals and Alternatives in Toxicity Testing," (Dewar, 1983). The FRAME report focuses on alternative techniques and enumerates methods by functional category: clinical observation and functional/behavioral tests; electrophysiological methods; neuropathological methods; biochemical methods; tissue culture methods; and tests in lower vertebrates and invertebrates. Several other reviews survey testing methods in this field, and include many methods that can be considered "alternative techniques" (Dewar and Moffett, 1979; Tilson and Mitchell, 1980; Dewar, 1980; Mitchell and Tilson, 1982; Tilson and Mitchell, 1984). These articles are authored by recognized experts who have conducted comprehensive, but not necessarily exhaustive, surveys of neurotoxicity testing methods.

A search of computerized data bases on the subjects of neurotoxicity and behavioral toxicity was conducted in order to build an extensive and current bibliography. The search was limited to the years 1979-1984, since the review articles thoroughly cover the prior period (as well as much of the more recent literature), and the goal was to identify all recent studies that might be useful as alternative methods. The bibliographic data bases searched on the National Library of Medicine and Dialog vendor systems were: Toxline, Medline, Excerpta Medica, Cancerlit, Life Sciences Collection, Biosis, Scisearch, NTIS, and Dissertation Abstracts.

Toxline was the most productive for this subject, and subsequent efforts were concentrated on this bibliographic data base. Data bases dealing with research proposals (SSIE and CRIS/USDA) and the Conference Papers Index were also searched. A truly exhaustive search could include many other vendors and their component data bases. There are diminishing returns as the search is extended, however, and computer searching is expensive. With limited resources, the searcher must decide what constitutes an adequate retrieval of information for the requirements of the project.

The Chemical Substances Information Network (CSIN) was employed for the execution of this search. CSIN is not a vendor for databases, but rather a "data manager". It provides access to a wide variety of vendor systems and their component data bases through a single source. CSIN is a particularly useful system for novice data base users, employing an interactive "menu" format of commands. It is very "user friendly." However, its ease of use reduces its flexibility, and limits the applications that are possible with direct use through individual data base vendors. Nevertheless, CSIN has many useful features available in its "enhanced direct" mode, which is basically a combination of direct searching with some features of the CSIN system.

The search strategy was to construct a list of related terms for each of the following: short-term or alternative assays, neurological science, and toxic effects, and to retrieve citations that scored at least one "hit" from each list. A trade-off occurs in the construction of these lists. The longer the list of terms searched, the more comprehensive but less selective the search. Consultation with an information specialist is recommended to ensure the design of an efficient search,

sufficiently broad but with a minimum number of "false drops."

The citation lists retrieved were screened manually for reports describing experiments or tests that have potential as alternative methods (not necessarily mentioned as such by the authors); reports whose thrust is the development of alternative toxicity tests; reports that analyze the validity of alternative tests, or correlate the results of an alternative toxicity test with standard test results; or reports of test applications - i.e., the use of existing alternative tests to assess the toxicity of chemicals.

Once reports had been identified as applicable to alternative testing for neurotoxicity or behavioral toxicity, they were organized into categories similar to those described in the FRAME report. These citations are listed in the bibliography in the following categories: clinical/epidemiological studies; behavioral/functional observations; electrophysiological or neuropathological methods; biochemical methods; tissue culture methods; lower vertebrate and invertebrate studies; and computer models and structure - activity relationship studies. As can be seen from these lists, this search retrieved a large number of studies related to behavioral toxicity, probably reflecting the level of interest in this subject in the scientific community (but may also be partially due to a bias in the chosen search strategy).

Several problems were identified that might be commonly encountered in information access and collection. A few of these have been mentioned in the preceding discussion, and generic problems were discussed in the Policy Issues section of the present report. A few specific problems related to this particular search will be mentioned.

The data bases that were searched provided varyingly useful information.

Toxline was the best journal literature data base for a survey of toxicity tests. Medline, Excerpta Medica, Life Sciences Collection, and the others mentioned earlier did not cover this particular field nearly as well, and seldom produced information that had not already been retrieved on Toxline. The Conference Papers Index was difficult to screen because abstracts are not included in this database. That is unfortunate because conference reports, although often not peer-reviewed, represent the most current research results of scientific investigators. The research proposal data base, SSIE, is completely out-of-date, inasmuch nothing has been added to it since 1982. CRISP and CRIS/USDA, which contain exclusively grant proposals supported by NIH and USDA respectively, are useful for identifying the research goals and directions of investigators, but do not provide actual research accomplishments. The frequency of updating individual data bases varies, and they are typically several months behind. To include very recent information, then, a computer search should be supplemented with a manual search.

Meetings on Neurotoxicity and Related Topics

Second International Symposium on Nephrotoxicity, Guilford, England, Aug. 6-9, 1984.

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Development of monitoring system for effects of chemicals on home cage behaviors. New York University Medical Center, Project No-1-ES-2-5017.

Food and Drug Administration

Strategies for neurobehavioral toxicity testing. Bureau of Foods, Division of Toxicology, Project No. 08855.

Neurobehavioral parameters sensitive to changes in membrane permeability. Bureau of Foods, Division of Toxicology, Project No. 08855.

The effects of chemicals and drugs on intracranial pressure, cerebrospinal fluid dynamics, brain edema and the blood-brain barrier. National Center for Drugs and Biologics.

Low-level chemical threat program. National Center for Toxicological Research, NCTR #6225.

Characterization of catecholaminergically-mediated changes in the swimming immobilization response in developing rats. National Center for Toxicological Research, NCTR #6231.

Collaborative study in behavioral teratology. National Center for Toxicological Research, NCTR #255, 285.

Analysis of d-amphetamine sulfate and methylmercury chloride. National Center for Toxicological REsearch, NCTR #286, 244.

Investigation of neurotoxic effects of drug exposure during development. National Center for Toxicological Research, NCTR #6115.

Study in behavioral teratology. National Center for Toxicological Research, NCTR #6127.

National Institute of Mental Health

Stimulant drug induced psychosis. University of Pittsburgh, Project No. 5-R01-MH24714-08.

Studies of neuroleptic induced tardive dyskinesia. Chicago Medical School, Project No. R01-MH33991-03.

Studies of neuroleptic induced tardive dyskinesia. New York State Psychiatric Institute, Project No. R01-MH33946-02.

Studies of dyskinesias produced by antipsychotic drugs. Texas Research of Institute of Mental Sciences, Project No. R01-MH334692--03.

Mechanisms of tardive dyskinesia. University of Michigan, Project No. R01-MH36044.

Long-term study of tardive dyskinesia. Medical Research Foundation of Oregon, Project No. R01-MH36657.

Behavioral toxicity. University of Rochester, Project No. R01-MH31850-04.

Environmental Protection Agency

In vivo short-term response indicators development. Health Effects Research Laboratory, HERL/A109C0501.

In vivo, in vitro biochemical test systems. Health Effects Research Laboratory, HERL/E104B0904.

In vivo, in vitro methods to detect neurotoxicity. Health Effects Research Laboratory, HERL/L104B0711

In vivo evaluation of significance of neurotoxic response indicators. Health Effects Research Laboratory, HERL/L104G0106.

In vitro, in vivo bioassay prescreening of hazardous wastes. Health Effects Research Laboratory, HERL/D109A0101.

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Department of Agriculture

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Bowen, J. M. Mechanisms of viral and environmental toxicant effects in cell culture. University of Georgia, Project No. GEOV-0161.

Chambers, H. W. Biochemical toxicology of insecticides: selectivity and resistance. Mississippi State University, Project No. MIS-6303.

Barthalmus, G. T., Tilson, H. A. Behavioral toxicology of herbicides. North Carolina State University, Project No. NC03668.

Appendix B[1]

Existing Laws Related to the Use of Animals in Biomedical Research

Animal Welfare Act

Congress passed the Animal Welfare Act in 1966 (P.L. 89-544) to regulate interstate trade in dogs and cats procured for research purposes. The Act was amended in 1970 (P.L. 91-579) to include most warm-blooded animals used in research, exhibitions, and in the wholesale pet trade. A second amendment in 1976 (P.L. 94-279) further extended coverage of the Act to include the transport of live animals. Regulations promulgated pursuant to the Act (as amended) established minimum standards for the care and treatment of dogs, cats, hamsters, guinea pigs, rabbits, and non-human primates (monkeys, apes, etc.) held by certain research facilities. When the Animal Welfare Act was first amended in 1970, the definition of "animal" was broadened to include "such other warm-blooded animal, as the Secretary may determine is being used, or is intended for use, for research, testing, experimentation, or exhibition purposes or as a pet." By regulation [9 CFR 1.1 (n)], some animal species, namely birds, rats, mice, horses, and other farm animals, are specifically excluded under the term "animal" and are considered administratively exempt from inclusion in annual reports that registered research facilities must file with the Department of Agriculture. At the present time, the Department is requesting laboratories to voluntarily report their use of rats and mice.

Department of Defense Appropriation Authorization Act of 1975

Title VII of this Act (P.L. 93-365) contains a general provision (sec. 703) placing certain restrictions but not a complete prohibition upon the use of dogs for chemical and other toxic substance research within the Department of Defense.

Supplemental Appropriation Act for Fiscal Year 1982

The Supplemental Appropriation Act (Public Law 97-257) was amended to add an expression of the sense of Congress that certain federal agencies should be encouraged to develop and validate an alternative to the Draize test. The Draize test is a controversial eye irritation test performed on laboratory rabbits.

Legislation

98th Congress

H.Con.Res. 58 (Jacobs et al.)

Expresses the sense of the Congress that when a federal agency utilizes the Draize rabbit eye irritancy test it should develop and validate

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[1]Most of this appendix was excerpted from: Randall, B. 1984. The use of animals in biomedical research. Issue Brief no. IB83161, Congressional Research Service. Two other sources were used: Griffin and Sechzer (1983), and Zola et al. (1984).

alternative ophthalmic testing procedures that do not require the use of animal test subjects. Introduced Feb. 15, 1983; referred to Committee on Energy and Commerce.

H.Res. 170 (Lantos et al.)

Commends Mobilization for Animals for its effort in protecting animals used in laboratory experiments. Introduced Apr. 21, 1983; referred to Committee on Post Office and Civil Service.

H.R. 2350 (Waxman)

Health Research Extension Act of 1983. Amends the Public Health Service Act relating to the National Institutes of Health (NIH) and the national research institutes, and has other purposes.

Three sections of this legislation are important to the issue of using animals in biomedical research. As proposed, new section 402(e) (1) of the Act would authorize the director of NIH to establish a plan for research into experimental methods that would not require the use of animals. This section also calls for finding new methods that would reduce the numbers of animals used in research, as well as finding new methods that would produce less pain and distress in such animals.

Proposed new section 487 of the Act would require the Secretary of the Department of Health and Human Services to issue guidelines concerning the care and treatment of animals used in biomedical research. These guidelines would also require each research entity receiving funding under the Act to have an animal care committee.

Section 5 of the bill would require the Secretary of DHHS to contract with the National Academy of Sciences to produce a study on the issue of using animals in biomedical research.

Introduced as H.R. 1555 Feb. 17, 1983; referred to Committee on Energy and Commerce. Hearings held by Subcommittee on Health and the Environment Feb. 23, 1983. Subcommittee approved bill with amendments, March 23. Clean bill (H.R. 2350) forwarded to full Committee in lieu. Committee consideration and markup held May 3, 5, and 10, 1983. Reported with amendments favorably, May 16, 1983 (H.Rept. 98-191). Considered in House July 25, 1983. Passed House, amended, Nov. 17, 1983. House incorporated H.R. 2350 as an amendment in the nature of a substitute to S. 540, June 5, 1984.

H.R. 2633 (Donnelly et al.)

Authorizes the Secretary of Health and Human Services to make grants for research and development of new methods of research, experimentation, and testing which minimize the use of, and pain and suffering to, live animals. Introduced Apr. 20, 1983; referred to more than one committee.

H.R. 5098 (Torricelli)

Promotes the dissemination of biomedical information through modern methods of science and technology and prevents the duplication of experiments

on live animals, and has other purposes. Introduced Mar. 8, 1984; referred to Committee on Energy and Commerce.

H.R. 5725 (Brown, G.)

Amends the Animal Welfare Act to ensure the proper treatment of laboratory animals. Requires that research facilities provide assurances satisfactory to the Secretary of Agriculture that a principal investigator has considered alternatives to any procedure likely to produce pain or distress in an experimental animal and shall provide details of any procedure likely to produce pain or distress in any experimental animal. Requires each research facility to establish an animal research committee which shall be made up of no less than three members possessing sufficient ability to assess animal care, treatment, and practices in experimental research. Provides assurances that members of the animal research committee do not release any confidential information of the research facility. Directs the Secretary to establish an information service at the National Agriculture Library to provide information on improved methods of animal experimentation. Introduced May 24, 1984; referred to Committee on Agriculture.

S. 657 (Dole et al.)

Amends the Animal Welfare Act to revise the humane standards for animals transported in commerce. Requires each research facility to establish an institutional animal studies committee with sufficient expertise to assess the appropriateness of animal care and treatment in experimental research. Directs the Secretary of Agriculture to establish an information service at the National Agricultural Library to provide information on improved methods of animal experimentation. Introduced Mar. 2, 1983; referred to Committee on Agriculture, Nutrition and Forestry.

S. 773 (Hatch)

Animal Research Study Act of 1983 requiring an 18-month study of the use of animals in research. Bill added as amendment to NIH renewal authorization. Introduced Mar. 11, 1983; referred to Committee on Labor and Human Resources. Reported with amendment (S.Rept. 98-110) May 16, 1983.

H.R. 4185 (Addabbo)

Makes appropriations for the Department of Defense for FY84, and has other purposes.

As the DOD appropriations bills were being marked up and amended, language was added that prohibits the purchase of dogs nd cats to be used in training medical students or other personnel in surgical or other medical treatment of wounds produced by any type of weapon. The amendment evolved from the discovery that the Department of the Army was proposing to shoot anesthetized, pound-acquired dogs for medical wound treatment training. The legislation, as amended, passed the House November 2 and the Senate Nov. 8, 1983; House and Senate agreed to final language on Nov. 18, 1983. The bill was presented to the President on Nov. 29, 1983, and became P.L. 98-212 on Dec. 8, 1983.

97th Congress

H. R. 220 (Ferraro) (identical to H. R. 2210)

The Humane Methods of Research Act. Promoted the development of methods of research, experimentation, and testing that minimize the use of and pain and suffering to live animals. Authorized $60 million over a five-year period to study and utilize alternative methods of research. No action taken.

H. R. 556 (Roe, Hollenbeck, and Richmond)

The Research Modernization Act. Sought the establishment of a National Center for Alternative Research, to develop and coordinate alternative methods of research and testing which do not involve the use of live animals, to develop training programs in the use of live animals, to eliminate or minimize the duplication of experiments on live animals, to disseminate information on such methods. It would have diverted 30-50% of federal funds for all research and testing programs that involve the use of live animals to establish the Center. No action taken.

H. R. 930 (Weiss)

The Protection of Animals in Research Act. Sought to establish an 11-member commission to study alternative methods to the use of live animals in laboratory research and testing, using appropriations not to exceed $750,000 per year for five years. No action taken.

H. R. 4406 (Schroeder)

To Amend the Animal Welfare Act to Insure the Humane Treatment of Laboratory Animals. Proposed improved standards for the use of live animals in research facilities, would have expanded the Act to include any vertebrate animal, and would have mandated procedures for the elimination or reduction of pain. No action taken.

H. R. 6245 (Walgren)

The Humane Care and Development of Substitutes for Animals in Research Act. Introduced in April 1982, after hearings on the above bills and combined several features of these. It sought the development of non-animal methods of research, experimentation, and testing, and the humane care of animals used in scientific research, experimentation, and testing. It provided for the accreditation of research facilities by a private agency. Its orginal language provided for the appropriation of $45 million in new funds over three years. It was later amended to eliminate the provision of new funds for alternative research, to introduce a threshold number of animals in research facilities before accreditation was required, and to totally review the law after ten years. A clean bill reissued as H. R. 6928 on August 4, 1982. A nearly identical version of the bill was introduced in the Senate by Sen. Dole as S. 2948, the major difference being that in the Senate version an advisory panel would report on the impact of the legislation three years after enactment. S. 2948 was amended and renumbered to S. 3630. Hearings were held in the House in December 1982, and changes in H. R. 6928 were made to make it consistent

with S. 3630, but Subcommitte members could not agree on all the changes, and no action was taken on either bill by the close of the 97th Congress.

Hearings

98th Congress

U. S. Congress. House Committee on Agriculture. Subcommittee on Department Operations, Research, and Foreign Agriculture. Current Enforcement of the Animal Welfare Act; and H. R. 5725, Improved Standards for Laboratory Animals Act. Hearings, 98th Congress, 2nd session, Sept. 19, 1984.

U.S. Congress. Senate Committee on Agriculture, Nutrition, and Forestry. Improved Standards for Laboratory Animals. Hearings, 98th Congress, 1st session, on S. 657. July 20, 1983. Washington, U.S. Govt. Print. Off., 1984.

97th Congress

U.S. Congress. House Committee on Energy and Commerce. Subcommittee on Health and the Environment. Humane Care and Development of Substitutes for Animals in Research Act. Hearings, 97th Congress, 2d session, on H.R. 6928. Dec. 9, 1982. Washington, U.S. Govt. Print. Off., 1982.

U.S. Congress. House Committee on Science and Technology. Subcommittee on Science, Research and Technology. Humane Care and Development of Substitutes for Animals in Research Act. Hearings, 97th Congress, 2d session, on H.R. 6245. May 4, 1982. Washington, U.S. Govt. Print. Off., 1982.

U.S. Congress. House Committee on Science and Technology. Subcommittee on Science, Research and Technology. The use of animals in medical research and testing. Hearings, 97th Congress, 1st session, on H.R. 556, H.R. 4406, and related bills. Oct. 13 and 14, 1981. Washington, U.S. Govt. Print. Off., 1981.